carried out with a double-stranded ribonuclease, such as RNAseH or Exo III. RNA probes made entirely of RNA sequences are particularly useful because first, they can be more easily produced enzymatically, and second, they have more cleavage sites which are accessible to nicking or cleaving by a nicking agent, such as the ribonucleases. Thus, scissile probes made entirely of RNA do not rely on a scissile linkage since the scissile linkage is inherent in the probe.

[0187] In a preferred embodiment, when the scissile linkage is a nucleic acid such as RNA, the methods of the invention may be used to detect mismatches, as is generally described in U.S. Pat. Nos. 5,660,988, and WO 95/14106, hereby expressly incorporated by reference. These mismatch detection methods are based on the fact that RNAseH may not bind to and/or cleave an RNA:DNA duplex if there are mismatches present in the sequence. Thus, in the NA_1—R—NA_2 embodiments, NA_1 and NA_2 are non-RNA nucleic acids, preferably DNA. Preferably, the mismatch is within the RNA:DNA duplex, but in some embodiments the mismatch is present in an adjacent sequence very close to the desired sequence, close enough to affect the RNAseH (generally within one or two bases). Thus, in this embodiment, the nucleic acid scissile linkage is designed such that the sequence of the scissile linkage reflects the particular sequence to be detected, i.e. the area of the putative mismatch.

[0188] In some embodiments of mismatch detection, the rate of generation of the released fragments is such that the methods provide, essentially, a yes/no result, whereby the detection of the virtually any released fragment indicates the presence of the desired target sequence. Typically, however, when there is only a minimal mismatch (for example, a 1-, 2- or 3-base mismatch, or a 3-base delection), there is some generation of cleaved sequences even though the target sequence is not present. Thus, the rate of generation of cleaved fragments, and/or the final amount of cleaved fragments, is quantified to indicate the presence or absence of the target. In addition, the use of secondary and tertiary scissile probes may be particularly useful in this embodiment, as this can amplify the differences between a perfect match and a mismatch. These methods may be particularly useful in the determination of homozygotic or heterozygotic states of a patient.

[0189] In this embodiment, it is an important feature of the scissile linkage that its length is determined by the suspected difference between the target and the probe. In particular, this means that the scissile linkage must be of sufficient length to encompass the suspected difference, yet short enough the scissile linkage cannot inappropriately "specifically hybridize" to the selected nucleic acid molecule when the suspected difference is present; such inappropriate hybridization would permit excision and thus cleavage of scissile linkages even though the selected nucleic acid molecule was not fully complementary to the nucleic acid probe. Thus in a preferred embodiment, the scissile linkage is between 3 to 5 nucleotides in length, such that a suspected nucleotide difference from 1 nucleotide to 3 nucleotides is encompassed by the scissile linkage, and 0, 1 or 2 nucleotides are on either side of the difference.

[0190] Thus, when the scissile linkage is nucleic acid, preferred embodiments utilize from 1 to about 100 nucleotides, with from about 2 to about 20 being preferred and from about 5 to about 10 being particularly preferred.

[0191] CPT may be done enzymatically or chemically. That is, in addition to RNAseH, there are several other cleaving agents which may be useful in cleaving RNA (or other nucleic acid) scissile bonds. For example, several chemical nucleases have been reported; see for example Sigman et al., Annu. Rev. Biochem. 1990, 59, 207-236; Sigman et al., Chem. Rev. 1993, 93, 2295-2316; Bashkin et al., J. Org. Chem. 1990, 55, 5125-5132; and Sigman et al., Nucleic Acids and Molecular Biology, vol. 3, F. Eckstein and D. M. J. Lilley (Eds), Springer-Verlag, Heidelberg 1989, pp. 13-27; all of which are hereby expressly incorporated by reference.

[0192] Specific RNA hydrolysis is also an active area; see for example Chin, Acc. Chem. Res. 1991, 24, 145-152; Breslow et al., Tetrahedron, 1991, 47, 2365-2376; Anslyn et al., Angew. Chem. Int. Ed. Engl., 1997, 36, 432-450; and references therein, all of which are expressly incorporated by reference. Reactive phosphate centers are also of interest in developing scissile linkages, see Hendry et al., Prog. Inorg. Chem.: Bioinorganic Chem. 1990, 31, 201-258 also expressly incorporated by reference.

[0193] Current approaches to site-directed RNA hydrolysis include the conjugation of a reactive moiety capable of cleaving phosphodiester bonds to a recognition element capable of sequence-specifically hybridizing to RNA. In most cases, a metal complex is covalently attached to a DNA strand which forms a stable heteroduplex. Upon hybridization, a Lewis acid is placed in close proximity to the RNA backbone to effect hydrolysis; see Magda et al., J. Am. Chem. Soc. 1994, 116, 7439; Hall et al., Chem. Biology 1994, 1, 185-190; Bashkin et al., J. Am. Chem. Soc. 1994, 116, 5981-5982; Hall et al., Nucleic Acids Res. 1996, 24, 3522; Magda et al., J. Am. Chem. Soc. 1997, 119, 2293; and Magda et al., J. Am. Chem. Soc. 1997, 119, 6947, all of which are expressly incorporated by reference.

[0194] In a similar fashion, DNA-polyamine conjugates have been demonstrated to induce site-directed RNA strand scission; see for example, Yoshinari et al., J. Am. Chem. Soc. 1991, 113, 5899-5901; Endo et al., J. Org. Chem. 1997, 62, 846; and Barbier et al., J. Am. Chem. Soc. 1992, 114, 3511-3515, all of which are expressly incorporated by reference.

[0195] In a preferred embodiment, the scissile linkage is not necessarily RNA. For example, chemical cleavage moieties may be used to cleave basic sites in nucleic acids; see Belmont, et al., New J. Chem. 1997, 21, 47-54; and references therein, all of which are expressly incorporated herein by reference. Similarly, photocleavable moieties, for example, using transition metals, may be used; see Moucheron, et al., Inorg. Chem. 1997, 36, 584-592, hereby expressly by reference.

[0196] Other approaches rely on chemical moieties or enzymes; see for example Keck et al., Biochemistry 1995, 34, 12029-12037; Kirk et al., Chem. Commun. 1998, in press; cleavage of G-U base pairs by metal complexes; see Biochemistry, 1992, 31, 5423-5429; diamine complexes for cleavage of RNA; Komiyama, et al., J. Org. Chem. 1997, 62, 2155-2160; and Chow et al., Chem. Rev. 1997, 97, 1489-1513, and references therein, all of which are expressly incorporated herein by reference.